Stability of daptomycin in peritoneal dialysis solutions packaged in dual-compartment infusion bags

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Received 29 January 2015

Revised 21 July 2015

Accepted 23 July 2015

Published Online First

20 August 2015

ABSTRACT

Background Intraperitoneal daptomycin is used for the treatment of peritoneal dialysis (PD)-associated peritonitis caused by vancomycin-resistant bacteria. This study investigated the stability of daptomycin in a pH-neutral PD solution.

Methods Reconstituted solution of daptomycin was added to a total of nine PD bags to obtain a concentration of 20 mg/L and stored at 1 of 3 different temperatures: 4 or 25°C for up to 168 h or 37°C for up to 48 h. Stabilities were determined by visual inspection, pH measurement and high-performance liquid chromatography.

Results Daptomycin retained more than 90% of its initial concentration for 120, 72 and 12 h at 4°C, 25°C and 37°C, respectively. No physical precipitation or pH change was observed during the study.

Conclusions Daptomycin in a pH-neutral PD solution is physically and chemically stable for 120 and 12 h at 4°C and 37°C, respectively.

INTRODUCTION

Peritoneal dialysis (PD) is a home-based treatment for patients with severe chronic kidney diseases. Peritonitis is one of the most fearful complications associated with PD. More than half of the PD-associated cases are caused by Gram-positive bacteria.¹ The use of vancomycin is recommended as the first-line agent for the treatment of ampicillin-resistant enterococcus/streptococcus or methicillin-resistant *Staphylococcus aureus* peritonitis.² Recently, a number of vancomycin-resistant PD-associated peritonitis cases have been reported where daptomycin has been successfully used to treat such infections.^{3–6} Daptomycin is a highly protein-bound drug and therefore in theory after systemic administration it would not achieve therapeutic concentrations in PD fluids.⁷ Nevertheless, Goedecke *et al*⁸ showed that the intravenous administration of daptomycin to a patient on PD can yield therapeutic concentrations of daptomycin in blood as well as in peritoneal fluid. However, intraperitoneal use of daptomycin is the standard approach for the treatment of PD-associated peritonitis to achieve its high local concentration. However, limited information is available regarding the stability of daptomycin in different types of PD solutions.⁹ Stability studies of daptomycin in glucose-containing PD solutions revealed that it is not stable for more than 6 h at 37°C.^{10–11} This limits the use of admixtures containing daptomycin and glucose-based PD solutions in night-time

sessions as dwell time is likely to be greater than 6 h. Additionally, the stability of daptomycin in glucose-containing PD solution is reported to be 24 h at 4°C or 25°C.^{10–11} Therefore, patients living far away need to travel to the hospitals every day to get daptomycin–PD admixtures. The above-mentioned problems could be avoided if daptomycin is stable for an extended period of time in commercially available PD solution. The stability of daptomycin is pH dependent and therefore we investigated its stability in a pH-neutral PD solution (Balance) under three different storage conditions.

METHODS

Sample preparation

Daptomycin (Novartis Pharmaceuticals, Australia) was reconstituted with water for injection British Pharmacopoeia according to the manufacturer's instructions. The reconstituted solution was then injected through the only available medication port connected to the non-glucose compartment (1.25 L) of the Balance (Fresenius Medical Care, Japan) two-compartment PD bag to obtain a final concentration of 20 mg/L of daptomycin. This concentration of daptomycin is recommended by the International Society of Peritoneal Dialysis guidelines as a maintenance dose in patients with PD-associated peritonitis.² After addition of daptomycin, the non-glucose compartment of the bag was gently shaken for approximately 1 min to ensure proper mixing. A total of nine pH-neutral PD bags containing daptomycin were prepared and stored at three different temperatures: 4°C in a domestic refrigerator, 25°C at room temperature and 37°C (body temperature) in an incubator. Control samples were prepared in an identical way except that the daptomycin was excluded. In clinical practice, immediately before the instillation of PD solution, the glucose-based solution from one compartment is mixed with the non-glucose solution present in another compartment. The PD bag is then warmed on a heating plate to attain the temperature of 37°C. Therefore, prior to the storage of a PD bag at 37°C, the glucose (1.5%)-based solution (1.25 L) from one compartment was mixed with the non-glucose solution present in the another compartment by opening the middle stream of the bag. After this, the solution was thoroughly mixed by gently shaking the bag for approximately 2 min. An aliquot (1 mL) from each bag was withdrawn and the concentration of daptomycin before and after storage was determined using a stability indicating high-performance liquid

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To cite: Ramdas S, Yousaf F, Shastri MD, *et al*. *Eur J Hosp Pharm* 2016;**23**:57–60.

chromatography (HPLC). Baseline samples (0 h) were kept at room temperature and subjected to HPLC analysis within 30 min of their preparation. Samples stored at 4°C or 25°C were analysed after 12, 24, 48, 72, 120 and 168 h. All samples stored at 37°C were analysed after 4, 8, 12, 24 and 48 h. Samples were also assessed for pH, colour and precipitation immediately after preparation and on each day of analysis.

HPLC instrumentation

Chromatographic analyses were performed on a HPLC system (Thermo Fisher Scientific, New South Wales, Australia) consisting of a HPG-3400 RS binary separation pump, TCC 3000RS column thermal compartment, WPS-3000TRS autosampler and VWD-3000RS diode array detector. Instrument control and data acquisition were performed using Chromeleon software.

HPLC assay

HPLC separation of daptomycin was carried out using a newly developed stability-indicating reversed-phase HPLC assay. Briefly, a Kinetex C₁₈ column (150 mm×4.6 mm×5 µm) with a 100 Å pore size (Phenomenex, Victoria, Australia) was used for the separation of daptomycin. Mobile phase A was composed of 10 mM ammonium acetate (adjusted to pH 5.5) and mobile phase B was composed of tetrahydrofuran in methanol at a ratio of 1:2. The gradient for mobile phase B was set as follows at 1.0 mL/min: 40%–60% over 0–5 min, 60% for 1 min, 60%–40% for 1 min and 40% for 3 min. The temperatures of the column and sample compartments were set at 30°C and 5°C, respectively. The injection volume was 25 µL and the detector was set at 262 nm.

Assay performance

Linearity

Calibration standards containing 5, 10, 20, 40 and 80 µg/mL of daptomycin were prepared by diluting daptomycin reference standard (Sigma-Aldrich, New South Wales, Australia) in mobile phase A. The relationship (over five consecutive days) between daptomycin concentration and detector response was determined by analysing calibration standards of daptomycin. Calibration graphs were generated by plotting peak areas versus daptomycin concentrations and used for the calculations of linear regression coefficient (r^2). Each sample was prepared in triplicate and analysed in triplicate.

Accuracy, precision and reproducibility

Four different concentrations of daptomycin standards (10, 20, 40 and 80 µg/mL) were used for the determination of accuracy and precision. Mean intraday and interday (five consecutive days) accuracy values for the daptomycin peak ($n=6$) were determined by the regression equation as follows: (observed concentration–expected concentration)/expected concentration×100. Intraday and interday (five consecutive days) precision values were investigated using peak areas with repeat analysis ($n=6$) of daptomycin. Reproducibility was investigated by determining mean intraday and interday (five consecutive days) peak retention time of daptomycin (20 µg/mL, $n=6$). Each sample was analysed in triplicate.

Forced degradation

The stability-indicating capability of the developed HPLC method was determined by subjecting daptomycin to an acidic, basic or oxidative stress in capped glass vials. The solution of daptomycin ($n=3$, 20 µg/mL) was mixed with an equal volume of 1 M hydrochloric acid, 1 M sodium hydroxide or 0.5%

hydrogen peroxide and then heated at 50°C for 30 min. HPLC analysis was performed in triplicate on stressed ($n=3$) as well as on unstressed samples ($n=3$).

Sample preparation for HPLC analysis

A standard stock solution of daptomycin (500 µg/mL) was prepared by dissolving 5 mg of daptomycin reference standard (Sigma-Aldrich, Victoria, Australia) in 10 mL of mobile phase. The standard stock solution was then diluted with mobile phase A to obtain a series of standard solutions (over the range of 5–80 µg/mL). A standard curve ($n=2$) was generated on each day of analysis by linear regression of the peak area of daptomycin against its concentration. An aliquot (1.5 mL) withdrawn from each PD bag was transferred to a glass autosampler vial and subjected to HPLC analysis within 30 min. Each sample was analysed in triplicate.

Data analysis

The initial concentration of daptomycin was considered as 100%. Its chemical stability was determined by calculating the percentage of the initial concentration remaining at each time period. Daptomycin was considered stable if it retained more than 90% of its initial concentration.

RESULTS

Stability-indicating capability of the newly developed HPLC method

To determine if the developed HPLC method was stability indicating, daptomycin was stressed under various conditions. Different chromatographic profiles were obtained for daptomycin when stressed under acidic, basic or oxidative conditions. With the unstressed sample, the peak of daptomycin eluted at 5.7 min. Exposure to hydrochloric acid 0.5 M, sodium hydroxide 0.5 M and hydrogen peroxide 0.25% for 30 min resulted in the loss of 18%, 37% and 60% of daptomycin, respectively. Importantly, the observed degradation products did not interfere with the peak of daptomycin, suggesting the developed HPLC method is suitable for the stability determination of daptomycin.

Assay performance

Linearity estimated by r^2 was greater than 0.999 with each of the six concentrations of daptomycin over 5 days. The intraday and interday precision relative SD (RSD) was less than 2.3% ($n=6$) and 2.9% ($n=6$), respectively, at the level of 10, 20, 40 and 80 µg/mL. The intraday and interday accuracy RSD at the level of 10, 20, 40 and 80 µg/mL was less than 2.6% ($n=6$) and 3.1% ($n=6$), respectively. Intraday and interday retention time RSDs for daptomycin (20 µg/mL, $n=6$) were less than 0.84% and 0.97%, respectively.

Stability of daptomycin

The percentage of the initial concentration of daptomycin remaining after storage at three different temperatures is shown in [figure 1](#). Daptomycin solution retained more than 95% of its initial concentration for 120 h at 4°C but lost more than 14% of its initial concentration after 168 h. Storage of daptomycin solution at 25°C retained more than 90% of its initial concentration for 72 h. Daptomycin was found to be stable for 12 h at 37°C but lost more than 10% of its initial concentration after 24 h of storage. The baseline pH of the daptomycin solution in the non-glucose compartment was 9.13 ± 0.3 or 9.16 ± 0.2 , whereas the baseline pH of daptomycin solution in the mix compartment (after mixing the non-glucose and glucose

Figure 1 The percentage of daptomycin (20 µg/mL) remaining in the Balance peritoneal dialysis (PD) solution before and after storage at 4°C, 25°C or 37°C for various time intervals. Results are presented as mean±SD. Samples were prepared in triplicate and analysed in triplicate.

compartment) was 8.23 ± 0.47 . As shown in [table 1](#), the pH of daptomycin solution remained essentially unchanged for 168 h when stored at 4°C or 25°C and for 48 h when stored at 37°C. No precipitation was observed under any of the storage conditions. However, the solution became pale yellowish in colour after 48 h at 37°C and 168 h at 25°C.

DISCUSSION

This is the first study that investigated the stability of daptomycin in a pH-neutral PD solution. The stability of daptomycin in glucose-containing PD solution has been reported to be 24 h at 4°C or 25°C and 6 h at 37°C before.^{10–11} The rate of daptomycin degradation is pH dependent and the appearance half-life of its degradation products at pH 4.5 and 25°C is 6 h, while the half-life is increased to 63 h at pH 7.0 and 25°C.¹² The pH of the commercially available glucose-PD solution is acidic

(4.9–5.2), where daptomycin is expected to be hydrolysed due to its multiple periodic bonds, and this may explain the limited stability of daptomycin in such types of PD solutions. The pH-neutral PD solution used in the current study is available in a two-compartment bag with a glucose-based electrolyte solution (pH 3.0) partitioned from the non-glucose solution (pH 8.3). Before peritoneal administration, the acidic and basic solutions partitioned in the two compartments are mixed together resulting in a pH-neutral solution. In the current study daptomycin was injected in the non-glucose compartment and PD bags were then stored at 25°C or 4°C, or both the compartments were mixed before storing them at 37°C. Daptomycin was found to be more stable compared with the previously reported studies, most likely because of the basic or neutral pH of the non-glucose or mixed solution.

Hospital pharmacists or PD nurses often refer to the available stability information to prepare antibiotic-containing PD solutions in advance in order to provide extra supply to the patients living far away from the hospitals or for those who are unable to self-load antibiotics into their own PD solutions. This study found that daptomycin in a pH-neutral PD solution is physically and chemically stable for at least 120 and 72 h when stored at 4°C and 25°C, respectively. Therefore, advance preparation of such admixtures in batches and appropriate storage would avoid the necessity for frequent preparation. Patients are often advised to warm PD solution to approximately body temperature prior to instilling into the peritoneal cavity. This enhances both the patient's comfort and removal of waste products when the dialysate fills the peritoneal cavity. Our results indicated that the pH-neutral Balance PD solution containing 20 mg/L of daptomycin was stable for 12 h and can be prewarmed to body temperature as well as can be used during night-time sessions where the dwell time exceeds more than 6 h.

In conclusion, this study provides crucial information to healthcare professionals on the physical and chemical stability of daptomycin in the pH-neutral Balance solution to help them in preparing such admixtures in advance where required.

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Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

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Table 1 The pH of daptomycin (20 or 40 µg/mL) peritoneal dialysis (PD) solution before and after storage at 4°C *, 25°C * or 37°C† for various time intervals

Time (hours)	pH		
	4°C	25°C	37°C
0	9.13±0.3	9.16±0.2	8.23±0.4
4	—	—	8.10±0.1
8	—	—	8.21±0.4
12	9.42±0.3	9.39±0.2	8.18±0.2
24	9.49±0.4	9.46±0.1	8.11±0.3
48	9.39±0.3	9.45±0.3	8.19±0.1
72	9.47±0.2	9.37±0.2	—
120	9.50±0.1	9.39±0.4	—
168	9.44±0.3	9.43±0.3	—

Results are presented as mean±SD. Samples were prepared in triplicate and analysed in triplicate.

*Concentration of daptomycin in PD solution was 40 µg/mL. Daptomycin was added to the non-glucose solution to obtain a concentration of 40 µg/mL before storing a PD bag at 4°C or 25°C. In clinical practice, both the solutions (glucose and non-glucose) are mixed together, to obtain a concentration of 20 µg/mL, before instilling it into the peritoneal cavity.

†Concentration of daptomycin in PD solution was 20 µg/mL. Glucose and non-glucose solutions were mixed and then daptomycin was added to obtain a final concentration of 20 µg/mL.

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